Synarc Assays

1. Biochemical Markers of Bone Turnover

1.1 Sample collection :

Measurements were performed on archived serum specimens.

1.2 Sample handling :

Specimens were sent frozen in one batch to Synarc using dry ice. Synarc sent a fax back to the repository lab to confirm the receipt of the shipment.

Upon arrival at Synarc, samples were rapidly stored at –70°C in freezers until assayed. The location of the samples was recorded in a logbook. Samples were kept in –70°C until the assay was performed. All –70°C freezers were identified by their own number and were connected with an alarm center in order to prevent the thawing of the samples. A calibrated thermometer was present in each freezer and refrigerator and the temperature was recorded manually or electronically each working day on a Temperature Log Sheet.

1.3 Quality control procedure for bone markers assays :

To ensure the quality of the analyses, equipment was periodically controlled and calibrated by the manufacturer. Freezers (-70°C and -20°C) were inspected once a year by the manufacturer. The pipettes used for the assays were checked and calibrated every 4 months by Gilson SAV. The maintenance log sheets and service reports were archived and are available for audit.

Samples were analyzed using reagents provided in commercial kits and according to standard operating procedures (SOPs) based on the manufacturer's recommendations. All samples originating from the trial were analyzed by the same reagents and according to the same SOPs. For each analysis in the laboratory, a GLP form named "Assay Information" was filled out.

In order to ensure the long-term precision of the measurements, one human control (3 for automatic analyzer) from the kit (high or low) and two human serum or urine pools were used as internal controls of quality. These internal controls were kept at -70°C and were measured in each assay during the entire study period. In general, the assigned value for the internal control was either the value from the manufacturer or a value determined by Synarc by analyzing the control several times over several days. Data were documented and archived with the experimental data. All samples were measured in duplicate for manual assays and once for automatic analyzers. If the CV of the duplicate was larger than the analytical intra-sample CV of the technique, the sample was re-assayed. All assays were crosschecked by two technicians or by the manager. Quality control information concerning the results of the assays was documented and archived in the binder QC

assays. Synarc also participates in an external calibration program specifically designed for bone markers and involving several laboratories in Europe (NEQAS, UK).

The following molecular markers of bone turnover were measured:

1.4 Serum bone alkaline phosphatase (bone ALP)

Serum bone ALP were measured by an immunochimiluminescence assay using the Ostase reagent on an automatic analyzer (Ostase, Access, Beckman Coulter). The intra- and inter-assay CVs were below 5 and 8%, respectively and the cross-reactivity with the liver isoenzyme was 13%.
The mean value (SD) for premenopausal women was: 9.04 (3.28) ng/ml.
The mean value (SD) for healthy untreated postmenopausal women was: 12.47 (5.19) ng/ml.

Reference range:

S-BAP	ng/ml	Nanogram per milliliter	**5.8 – 26.5**

1.5 Serum C-terminal cross-linking telopeptide of type I collagen (serum CTX):

Serum CTX was measured by a two-site assay using monoclonal antibodies raised against an 8 amino-acid sequence from the C-telopeptide of human type I collagen by an automatic analyzer (Elecsys, Roche Diagnostic). Intra-assay variation was lower than 3% and inter-assay variation was lower than 8%. The mean (SD) value for pre-menopausal women was 0.299 (0.137) ng/ml. The mean (SD) value for postmenopausal women was 0.556 (0.226) ng/ml.
Reference range:

S-CTX	ng/ml	Nanogram per milliliter	**0.087 – 1.150**

Ref: Garnero P , Borel O, Delmas PD. 2001. Evaluation of a Fully Automated Serum Assay for C-terminal Crosslinking Telopeptide of Type I collagen in Osteoporosis. Clin Chem; 47:694-702.

1.6 Serum Intact PTH :

Serum intact PTH was measured using a two site immunoassay (ELECSYS, Roche Diagnostics). This assay has no cross-reactivity with C- and N-terminal fragments, including the large 7-84 peptide. Intra assayvariation was lower than 5% and interassay variation was lower than 7%.

Reference range:

S-PTH	pg/ml	Picogram per milliliter	**15 – 65**

1.7 Serum calcium :

Serum calcium was measured by a standard colorimetric assay on a Kone specific automatic analyzer.

Reference range:

S-CA	mg/L	Milligram per liter	**87 - 106**

2. Data Handling & Data Management

The raw data included the printouts of the analysis apparatus. Each raw data set was identified by the specimen code, which includes protocol number, patient number, visit date, and requisition number.

Results were entered in a central relational database. The database was password protected and was on an internal network server that was password protected. This system allows a real-time backup of the data by copying them to 3 different hard disks (RAID 5 technology). Every night a backup of the data was made on cartridge placed in a fireproof cabinet. Weekly backup was stored offsite. From the database, it was possible to print laboratory reports (patient report) and extract data for external data transfer.

For manual assays, measurements of each sample were entered into the database by two different technicians; each blinded to the data entered by the other. If the second entered value was different from the first value, the value was checked against the original raw data report and corrected. For automatic assay, measurements were transmitted on line from the analyzer directly to the database.

3. Specimen Archival

Synarc stored all serum in –70°C freezers. A dedicated storage space within the freezer was provided. The location of the samples was recorded in the logbook. All –70°C freezers were identified by their own number and were connected with an alarm center in order to prevent the thawing of the samples. A calibrated thermometer was present in each freezer and the temperature was recorded manually or electronically each working day on a Temperature Log Sheet.